

Effects of mild alternate wetting and drying irrigation and rice straw application on N₂O emissions in rice cultivation

**Kaikuo Wu^{1,2}, Wentao Li^{1,3}, Zhanbo Wei^{1,2}, Zhi Dong^{4,5}, Yue Meng^{1,3},
Na Lv^{1,3}, Lili Zhang^{1,2,6}**

¹ Institute of Applied Ecology, Chinese Academy of Sciences, Shenyang
110016, China

² Engineering Laboratory for Green Fertilizers, Chinese Academy of
Sciences, Shenyang 110016, China

³ University of Chinese Academy of Sciences, Beijing 100049, China

⁴ Institute of Tillage and Cultivation, Liaoning Academy of Agricultural
Sciences, Shenyang 110161, China

⁵ Liaoning Key Laboratory of Conservation Tillage in Dry Land,
Shenyang 110161, China

⁶ National Engineering Laboratory for Soil Nutrient Management,
Shenyang 110016, China

Correspondence: Lili Zhang (llzhang@iae.ac.cn)

Abstract. The shortage of water resources and the decline in soil organic matter (SOM) are critical limiting factors affecting the improvement in rice productivity, while alternate wetting and drying (AWD) irrigation and recycling application of rice straw are considered favourable

mitigation measures. However, the impact of such measures on rice yield and greenhouse gas (GHG) emissions, especially nitrous oxide (N₂O) emissions, needs to be further clarified to ensure that agronomic practises save water, conserve soil, and reduce GHG. Therefore, we explored the effects of mild AWD irrigation combined with on-site rice straw recycling on N₂O emissions and rice yield through rice pot experiments. This experiment included two irrigation methods (continuous flooding (CF) irrigation and mild AWD irrigation), two nitrogen (N) application levels (0 and 225 kg N ha⁻¹) and two rice straw (S) return levels (0 and 9000 kg ha⁻¹), for a total of 10 treatments, and each treatment had three replicates. ¹⁵N-urea and ¹⁵N-S were added to the soil. The results showed that N₂O emissions were primarily affected by urea application and irrigation methods, with urea application being most important. Compared with CF irrigation, mild AWD irrigation increased cumulative N₂O emissions, with an average increase of 28.8%. In addition, adding rice straw to mild AWD irrigation further stimulated N₂O emissions by 18.1%. Under the condition of urea application, compared with CF irrigation, mild AWD irrigation increased the yield-scaled N₂O emissions by 17.9%, and the addition of rice straw further promoted the yield-scaled N₂O emissions under mild AWD irrigation by 17.4% but reduced the global warming potential (GWP) (methane (CH₄) + N₂O) by 62.9%. Under the condition of urea application, compared with CF irrigation, mild AWD irrigation

reduced the uptake of soil-derived N and aboveground biomass of rice but did not reduce rice yield. Therefore, mild AWD irrigation combined with rice straw return may be a promising agronomic method to maintain rice yield, reduce greenhouse gases, and protect or improve soil fertility.

1 Introduction

Rice is a staple food for more than half of the world's population and ensuring rice production is crucial to food security (Tang and Cheng, 2018). More than 135 million hectares of rice are cultivated worldwide, and approximately 90% of paddy fields are submerged (Wang et al., 2017a). Feeding a growing population under water scarcity will be a major challenge to Asia's food security in the coming decades (Lampayan et al., 2015). In China, more than 60% of freshwater resources are consumed by rice cultivation every year, which represents a great waste of freshwater and causes many environmental problems, such as nonpoint source pollution, eutrophication, and GHG emissions (Liao et al., 2020). Therefore, it is urgent to explore new methods for managing paddy field fertilization that can ensure high rice yield and reduced water waste and pollution.

AWD irrigation is an effective water-conserving irrigation method that can save approximately 23% of freshwater resources compared with CF irrigation (Bouman and Tuong, 2001; Chu et al., 2014). There are

usually two approaches to alternate wetting and drying irrigation: severe AWD irrigation (soil water potential $\geq -30 \pm 5$ kPa) and mild AWD irrigation (soil water potential $\geq -15 \pm 5$ kPa) (Zhou et al., 2017). Severe AWD irrigation could reduce rice yield by 22.6% due to water stress, but under mild AWD irrigation, rice yield can be stable or slightly increased (Carrijo et al., 2017; Zhou et al., 2017). Therefore, mild AWD irrigation may offer a more promising paddy field management model. Previous studies have shown that AWD irrigation can significantly reduce CH₄ emissions but considerably promotes N₂O emissions, while GWP mitigation is dependent on the magnitude of the increase in the release of N₂O (Lagomarsino et al., 2016; Liang et al., 2017; Kritee et al., 2018; Tan et al., 2018). Therefore, exploring the impact of mild AWD irrigation on GHG emissions, especially N₂O, is conducive to reducing paddy field emissions and maximizing agricultural and environmental benefits.

Long-term cultivation without organic matter supplementation leads to serious degradation of cultivated land and reduction in soil organic matter (SOM) content (Zhou et al., 2021), which is not conducive to rice production and sustainable agricultural development (Chen et al., 2016). Straw return to the field is a valuable measure for improving SOM (Huang et al., 2021), and it is beneficial for reducing the environmental pollution caused by burning straw or discarding it randomly (Wang et al., 2018). In addition, rice straw return may cause changes in paddy field

GHG emissions (Naser et al., 2007; Ye et al., 2017; Sun et al., 2018; Yu et al., 2021), N use efficiency (Liu et al., 2021), and rice yield (Chen et al., 2016; Ku et al., 2019). Although there have been many studies on mild paddy AWD irrigation or rice straw return, few studies have focused on the effects of mild AWD irrigation combined with rice straw return on rice cultivation. Under the conventional fertilization mode, whether mild AWD irrigation combined with rice straw return can achieve the optimal goals of water saving, yield increase, and reduction in greenhouse gas emissions has remained unclear. Therefore, the purpose of this study was to investigate the effect of mild AWD irrigation combined with rice straw return on N₂O emissions and rice yield in rice cultivation and to explore the supply of N to rice growth from the soil, urea, and rice straw using ¹⁵N labelling technology. Our initial hypotheses were that: 1) Mild AWD irrigation would promote N₂O emissions in rice cultivation; and 2) Mild AWD irrigation would maintain or promote rice yield.

2 Materials and methods

2.1 Experimental setup

A pot experiment was conducted in an open greenhouse at the Shenyang Experimental Station of the Institute of Applied Ecology, Liaoning Province, China (43°32'N, 123°23'E) from June 17th to October 27th, 2020. The test soil was an Alfisol with a total C content of 16.01 g/kg and a total N content of 1.36 g/kg.

The pot experiment used a random block design, including 30 pots (30 cm diameter $\times$ 20 cm height). This experiment included two irrigation methods, two N application levels, and two rice straw return levels, with three replicates of each combination, for a total of 30 rice pots (urea and straw were labelled with ^{15}N , respectively). The two irrigation methods were CF irrigation and mild AWD irrigation. CF irrigation maintained a water level depth of approximately 3-5 cm throughout the rice-growing season. Mild AWD irrigation water management in the first 7 days was consistent with CF irrigation and allowed to evaporate under monitoring; when the soil negative pressure gauge reached -15 kPa, it was sub-flooded to a depth of 3-5 cm again and then naturally allowed to dry again. This step was repeated until harvest. CF and mild AWD irrigation were halted 2 weeks before harvest. N was applied at 0 kg N ha $^{-1}$ (CK (control check) and ^{15}S (rice straw)) and 225 kg N ha $^{-1}$ (106.13 mg kg $^{-1}$ dry soil) (^{15}U (urea), U^{15}S (urea + rice straw) and ^{15}US (urea + rice straw)). The abundance of urea ^{15}N was 10.20%. Urea was applied three times: base fertilizer 40% (June 17th), tiller topdressing 30% (August 4th) and heading topdressing 30% (August 25th). Rice straw return was applied at 0 kg ha $^{-1}$ (CK and ^{15}U) and 9,000 kg ha $^{-1}$ (4.25 g kg $^{-1}$ dry soil) (^{15}S , U^{15}S and ^{15}US). The total N content of unlabelled rice straw was 0.72%, and the isotope abundance of ^{15}N was 0.59%. The total N content of labelled rice straw was 0.73%, and the ^{15}N isotope abundance was

22.94%. The rice straw was ground and applied together with the base fertilizer. Phosphate fertilizer was superphosphate ($150 \text{ kg P}_2\text{O}_5 \text{ ha}^{-1}$), and potassium fertilizer was potassium chloride ($185 \text{ kg K}_2\text{O ha}^{-1}$) as a one-time application of basic fertilizer. Every pot was filled with 10.51 kg (9 kg dry soil) of sieved (2 mm) fresh soil. Two hills of rice were planted in each pot. At maturity, the rice yield and aboveground biomass were recorded after being oven dried (105°C for 0.5 h and 60°C for 12 h).

2.2 Soil sample collection and analysis

At the regreening stage, tillering stage, jointing stage, booting stage, filling stage, and maturity stage, five points were randomly selected from the 0-10 cm soil layer of each pot and mixed. The soil $\text{NH}_4^+\text{-N}$ and $\text{NO}_3^-\text{-N}$ were extracted with 2 mol L^{-1} KCl solution (Wu et al., 2019), filtered and analysed with a continuous flow analyser (AA3, Bran + Luebbe, Germany). The extraction of soil $^{15}\text{N-NH}_4^+\text{-N}$ followed Yu et al. (2020). Soil microbial biomass N (MBN) was fumigated with chloroform, extracted with 0.5 mol L^{-1} K_2SO_4 (soil: solution = 5 g: 20 ml) (Joergensen et al., 1996), and determined by a TOC analyser (Elementar vario TOC Analyzer, Germany). The soil $^{15}\text{N-NH}_4^+\text{-N}$ content, $^{15}\text{N-MBN}$ and ^{15}N of rice aboveground biomass were determined by a stable isotope ratio mass spectrometer (253 MAT, Thermo Finnigan, Germany).

2.3 Gas sampling and calculation

The static chamber method was used to determine the N_2O flux (Li

et al., 2018a). The static chamber with a top seal made of transparent plexiglass consisted of two parts, namely, the base and the gas collecting chamber. The base had a diameter of 31 cm, a groove in the middle, and a height of 10 cm. The gas-collecting chamber had a diameter of 30 cm and a height of 70 cm. A small fan and a thermometer were installed in the gas-collecting chamber. N₂O was collected every two days in the first week after fertilization or irrigation and every seven days during other periods. N₂O was sampled at 8:00–11:00 a.m. each sampling day. Every pot was sealed with water when N₂O was collected. Three gas samples were collected at 0, 30 and 60 min after the chamber was airtight, and N₂O was collected with a 50 mL injector and then injected into 200 mL gasbags.

The N₂O concentration was analysed using a gas chromatograph (Agilent 7890B, Gas Chromatograph, Delaware, USA). The calculation of N₂O fluxes was as follows (Li et al., 2018a):

$$F = \rho \times h \times dc/dt \times 273/(273 + T)$$

where F is the N₂O flux ($\mu\text{g m}^{-2} \text{ h}^{-1}$); ρ is the N₂O standard-state density (1.964 kg m^{-3}); h is the chamber height above the soil (m); c is the N₂O concentration; dc/dt is the slope of the N₂O concentration curve, estimated using a linear regression model (Vitale et al., 2017); 273 is the gas constant; and T is the average air temperature inside the chamber during N₂O collection (°C).

Cumulative N₂O emissions (CE) were calculated using the following formula according to Wang et al. (2011):

$$CE (kg N_2O ha^{-1}) = \sum_{i=1}^n \left(\frac{F_i + F_{i+1}}{2} \right) (t_{i+1} - t_i) \times 24 \times 10^{-2}$$

where i is the various sampling times, t is the sampling date, n is the total measurement time and 10^{-2} is the conversion factor.

The contribution of ¹⁵N markers to NH₄⁺-N and MBN and the calculation of the N source of the aboveground biomass of rice followed Ma et al. (2015). Yield-scaled N₂O was calculated as the ratio between N₂O and rice yield (Li et al., 2018a).

2.4 Statistical analysis

All analyses were performed using SPSS Statistics 16.0 (SPSS, Inc., Chicago, USA). One-way ANOVA was conducted to test the treatment effects with Duncan's test. Significant differences were set at alpha = 0.05. Univariate analysis of variance was used to analyse the response of cumulative N₂O emissions to irrigation method, N level and rice straw application (Table 2). Tables and figures were prepared with Excel 2016 (Microsoft Corp., USA) and Origin 8 (Origin Lab Corp., USA), respectively. The data in the figures and tables are the average value ± standard error.

3 Results

3.1 N₂O flux

Three higher N₂O flux peaks appeared after basal fertilizer and two topdressing treatments (Figure 1), and the N₂O flux peak after basal fertilizer application was significantly larger than the last two peaks. After basal fertilizer was applied, the N₂O flux peaks of CF irrigation and mild AWD irrigation were similar. After the first topdressing, the N₂O flux peak of mild AWD irrigation was significantly greater than that of CF irrigation, approximately 1.4 and 9.1 times under the U and US treatments, respectively. In contrast, the N₂O flux peak of CF irrigation after the second topdressing was 3.5 and 1.6 times higher than that of mild AWD irrigation under the U and US treatments, respectively. In addition to the above three peaks, the N₂O flux of CF irrigation was close to zero, and mild AWD irrigation had a lower flux peak with alternating wet and dry conditions. The flux of N₂O ranged from -103.93 to 2,770.50 $\mu\text{g m}^{-2} \text{ h}^{-1}$. The N₂O flux appeared negative in the late stage of rice growth. The N₂O fluxes of CF irrigation and mild AWD irrigation were similar in the later stage of rice growth, indicating that drainage had little effect on it. During the entire rice growth cycle, the N₂O flux of the CK and S treatments was low. There were significant differences in the peak N₂O fluxes between the different treatments. Compared with CK, the application of rice straw alone significantly promoted the N₂O flux on the first day after CF irrigation and mild AWD irrigation. Compared with U, the addition of rice straw in CF irrigation reduced the N₂O flux, while the

addition of rice straw in mild AWD irrigation increased the N₂O flux.

3.2 Soil NH₄⁺-N, NO₃⁻-N and MBN concentrations

The soil NH₄⁺-N, NO₃⁻-N, and MBN concentrations varied with the growing stage of rice (Fig. 2A, 2C and Fig. 3A). The soil NH₄⁺-N concentration first increased and then decreased. The NH₄⁺-N concentration under CF irrigation and mild AWD irrigation was low in the late rice growth period (Fig. 2A). The concentration of NO₃⁻-N in CF-irrigated soil showed a trend of first increasing and then decreasing and was at a low level in the later growth period of rice, while the concentration of NO₃⁻-N in mild AWD irrigation showed a trend of first decreasing and then increasing, with repeated decreases and increases (Fig. 2C). The concentration of MBN in CF-irrigated soil first decreased and then increased and then decreased to a lower level, while the concentration of MBN in mild AWD-irrigated soil decreased with the growth stage of rice (Fig. 3A).

There were significant differences in the NH₄⁺-N, NO₃⁻-N, and MBN concentrations between the different treatments. In CF irrigation and mild AWD irrigation, the U treatment had a higher NH₄⁺-N concentration in the early stage of rice growth, while the other treatments had no significant difference, but as rice grew, the NH₄⁺-N concentration of the US treatment increased, which was significantly greater than that of the U, S and CK treatments (Fig. 2A). In CF irrigation, the NO₃⁻-N

concentration of the U treatment was slightly higher than that of the other treatments, and all treatments had little variance. In mild AWD irrigation, the NO_3^- -N concentration of the US treatment was significantly higher than that of the U, S and CK treatments. The U treatment had a higher NO_3^- -N concentration than the S and CK treatments in the later stage of rice growth (Fig. 2C). The US treatment in CF irrigation and mild AWD irrigation had the highest MBN concentration during the rice growth period (Fig. 3A).

Figure 2B shows that the NH_4^+ -N in CF irrigation and mild AWD irrigation mainly came from urea rather than rice straw, and the combined application of urea and rice straw further promoted the release of NH_4^+ -N from urea. Regardless of CF irrigation or mild AWD irrigation, rice straw N was difficult to utilize by microorganisms in the first year under single rice straw application, but rice straw and urea combined application significantly promoted the utilization of rice straw N by microorganisms. Urea combined with rice straw application may be more easily utilized by microorganisms than urea applied alone (Fig. 3B).

3.3 Sources of aboveground biomass N in rice

As shown in Fig. 4, under CF irrigation and mild AWD irrigation, compared with CK, a single application of rice straw did not increase the aboveground N absorption of rice, while the U and US treatments significantly promoted the aboveground N absorption of rice. Under mild

AWD irrigation, the US treatment reduced N uptake in rice shoots compared with the U treatment. The U and US treatments under CF irrigation promoted the N uptake of the aboveground rice more than those under mild AWD irrigation.

With different irrigation methods, the effects of urea and rice straw addition on the N absorption of the aboveground rice were varied. Compared with the CK and S treatments, the U and US treatments under CF irrigation significantly promoted the absorption of soil N by rice, while only the S and U treatments had significant differences under mild AWD irrigation. Compared with mild AWD irrigation, the U and US treatments under CF irrigation significantly promoted the absorption of soil N by rice. Regardless of the irrigation and fertilization method, the soil was the main source of N in the aboveground parts of rice, followed by urea and finally rice straw (Fig. 4).

3.4 Cumulative N₂O emissions, rice agronomic properties and yield-scaled N₂O emissions

In addition to the CK treatment, compared with CF irrigation, mild AWD irrigation significantly promoted the accumulation of N₂O during the rice growth period, with an average increase of 28.8% (Table 1). Under CF irrigation, there was no significant difference in the accumulation of N₂O between S and CK or between US and U. However, the addition of rice straw under mild AWD irrigation significantly

increased the accumulation of N₂O by 18.1% (Table 1). Compared with the CK and S treatments, the U and US treatments significantly promoted cumulative N₂O emissions under the two irrigation modes. As shown in Table 2, irrigation methods, N application level, and rice straw return affected cumulative N₂O emissions, of which the N application level had the greatest impact. The interaction between irrigation level and N fertilizer or rice straw significantly affected cumulative N₂O emissions.

As shown in Table 1, compared with CF irrigation, mild AWD irrigation significantly reduced rice aboveground biomass under US treatment but had no effect on other treatments. Regardless of whether CF irrigation or mild AWD irrigation was applied, there was no significant difference in the rice aboveground biomass between S and CK or between US and U. Compared with the CK and S treatments, the U and US treatments significantly promoted the rice aboveground biomass under the two irrigation modes. Irrigation level had no effect on rice yield under all treatments. Regardless of CF irrigation or mild AWD irrigation, rice yield under the U and US treatments was significantly higher than that under the CK and S treatments, but there was no difference between the former two and the latter two.

In addition to the CK treatment, compared with CF irrigation, mild AWD irrigation significantly promoted yield-scaled N₂O emissions during the rice growth period. Under urea application conditions,

compared with CF irrigation, mild AWD irrigation increased yield-scale N_2O emissions by 17.9%, and the addition of rice straw further promoted yield-scale N_2O emissions by 17.4% under mild AWD irrigation conditions (Table 1). Regardless of CF irrigation or mild AWD irrigation, yield-scaled N_2O emissions under the U and US treatments were significantly higher than those under the CK and S treatments. Rice straw addition had no effect on the yield-scaled N_2O emissions under CF irrigation but significantly increased the yield-scaled N_2O emissions under mild AWD irrigation.

4 Discussion

4.1 Effects of irrigation methods, N levels, and rice straw return on N_2O emissions

N_2O emissions are significantly affected by water-filled pores and mineral N ($\text{NH}_4^+\text{-N}$ and $\text{NO}_3^-\text{-N}$) content (Allen et al., 2010). The N_2O emission peak in CF irrigation occurred only after N application, while mild AWD irrigation caused other N_2O emission peaks, which might have been caused by the change in soil moisture conditions by mild AWD irrigation (Zhou et al., 2020). The peak of N_2O after fertilization may be because a large amount of N application increases the soil inorganic N concentration (Fig. 2A and 2C), which in turn promotes the generation of N_2O , which comes from the denitrification process (Wang et al., 2017b; Yano et al., 2014). During the denitrification process, it is easier for

microorganisms to use NO_3^- -N as an electron acceptor (Fig. 2C), which affects the reduction process of N_2O , resulting in an increase in the ratio of $\text{N}_2\text{O}/\text{N}_2$ in the denitrification products (Pérez et al., 2000). Our results showed a negative N_2O emission flux at the later stage of rice growth, which may be due to the decrease in surface soil N_2O concentration due to the strengthening of the N_2O reduction process or the weakening of the N_2O diffusion process in the soil profile, which allowed atmospheric N_2O to diffuse back into the soil (Chapuis-Lydie et al., 2007). Mild AWD irrigation promoted cumulative N_2O emissions by 28.8% on average, which was as proposed in our Hypothesis 1. Similar results were found in previous studies, which may be due to the increased N_2O produced by nitrification and denitrification due to water level alternation (Liang et al., 2017; Zhou et al., 2020) and temperature change (Wu et al., 2019) of mild AWD irrigation. To reduce N_2O emissions from paddy fields, researchers generally regulate N_2O production by optimizing N management (Liang et al., 2017), applying inhibitors to control the N supply rate of N fertilizers (Wu et al., 2019). In both CF and mild AWD irrigation, there was no obvious N_2O emission peak at the later growth stage of rice, which may have been due to the decrease in soil inorganic N content (Fig. 2A and 2C) and microbial biomass (Fig. 3A).

Compared with CK and S, N fertilizer application (U and US) significantly increased N_2O cumulative emissions and was the most

notable factor in N₂O generation (Table 2), mainly because N fertilizer application provided sufficient substrates for soil nitrification and denitrification to generate N₂O (Fiedler et al., 2017; Wu et al., 2021). The peak of N₂O emissions after base fertilizer application was larger than that after two topdressing, which might have been due to a higher N application rate and simultaneous nitrification and denitrification in initial flooding (Mathieu et al., 2006; Wang et al., 2017b). The peak of N₂O emissions after the two topdressing treatments with CF irrigation was similar, while the peak of N₂O emissions after the first topdressing treatment with mild AWD irrigation was significantly larger than that following the second topdressing treatment. This may be because the soil environment (temperature, moisture, etc.) changed little in long-term flooding under CF irrigation (Lagomarsino et al., 2016; Verhoeven et al., 2018; Congreves et al., 2019), while the variations in the soil environment of the two top dressings under mild AWD irrigation changed the utilization of fertilizer N by microorganisms (Fig. 3B). Therefore, reducing the amount of the first top dressing under mild AWD irrigation and maintaining flooding for approximately one week after fertilization may benefit N₂O reduction (Liao et al., 2020). The negative value of N₂O emissions appeared at the later stage of rice growth, indicating that the paddy field could also become a sink for N₂O (van Groenigen et al., 2015), which might be caused by the lack of N supply at the later stage.

Increasing the second topdressing might be beneficial for alleviating N deficiency (Liao et al., 2020).

Unlike the CF irrigation, the addition of rice straw under mild AWD irrigation conditions promoted N₂O emissions (Table 1), probably because the alternation of wet and dry conditions promoted the decomposition of rice straw (Andren et al., 1993; Buchen et al., 2016; Chen et al., 2016), which was beneficial to the growth of microorganisms (Fig. 3A), thus promoting the production of N₂O (Said-Pullicino et al., 2014; Wang et al., 2018; Wu et al., 2021). In addition, compared with U and S, US promoted microbial absorption of urea and rice straw N (Fig. 3B), which also proved that US treatment was more conducive to the growth of microorganisms.

4.2 Effects of irrigation methods, N levels, and rice straw return on rice production and yield-scaled N₂O emissions

The change in irrigation method did not cause differences in rice yield (Table 1), but under the US treatment, mild AWD irrigation significantly reduced the aboveground biomass of rice and the uptake of soil N by rice (Table 1 and Fig. 4), which was consistent with our Hypothesis 2. Previous studies have also shown that mild AWD irrigation can stabilize or increase rice yield. This may be because mild AWD irrigation can promote the transport of nutrients from stems and leaves to grains during the reproductive growth stage of rice while inhibiting

ineffective tillering and increasing the number of effective panicles, thereby reducing excessive vegetative growth of rice (Carrijo et al., 2017; Li et al., 2018b; Liao et al., 2020; Zhang et al., 2009). This may also be an important reason for the decrease in the uptake of soil N by rice under AWD irrigation. Urea application was a key factor in improving rice yield (Wang et al., 2017a) but also aggravated soil N uptake by rice, and soil was the largest source of N for rice in all treatments (Fig. 4). Compared with U, under CF irrigation and mild AWD irrigation, US reduced the uptake of soil-derived N by rice, and the trend was more obvious under mild AWD irrigation. Although the trend was not significant, rice straw return may be an effective way to maintain long-term soil fertility (Fig. 4).

In our study, mild AWD irrigation, urea application and rice straw return all increased yield-scaled N₂O emissions (Table 1), mainly due to improved soil aeration and increased inorganic N and rice straw decomposition, resulting in more N₂O production (Andren et al., 1993; Buchen et al., 2016; Chen et al., 2016; Lagomarsino et al., 2016; Fiedler et al., 2017; Verhoeven et al., 2018; Congreves et al., 2019; Wu et al., 2021). Although mild AWD irrigation had higher yield-scaled N₂O emissions than CF irrigation, the GWP (CH₄ + N₂O) under mild AWD irrigation was significantly lower than that under CF irrigation and decreased by 8.1%, 57.9%, 11.8% and 62.9% under CK, S, U and US,

respectively (Table S1). Therefore, mild AWD irrigation combined with rice straw return may be a promising agronomic measure that maintains rice yield, slows greenhouse effects (CO_2 emissions are not considered), and also reduces soil fertility consumption.

5 Conclusions

The effects of irrigation methods, N levels and rice straw return on N_2O emissions were explored through pot experiments using rice. We found that N_2O emissions were affected by urea application and irrigation methods, with urea application being the most important. Compared with CF irrigation, mild AWD irrigation increased cumulative N_2O emissions, with an average increase of 28.8%. In addition, adding rice straw to mild AWD irrigation further stimulated N_2O emissions by 18.1%. Under the condition of urea application, compared with CF irrigation, mild AWD irrigation increased the yield-scaled N_2O emissions by 17.9%, and the addition of rice straw further promoted the yield-scaled N_2O emissions under mild AWD irrigation by 17.4% but reduced the GWP ($\text{CH}_4 + \text{N}_2\text{O}$) by 62.9%. Under the condition of urea application, compared with CF irrigation, mild AWD irrigation reduced the uptake of soil-derived N and aboveground biomass of rice but did not reduce rice yield. Therefore, mild AWD irrigation combined with rice straw return may offer a promising agronomic measure to maintain high rice yield, reduce greenhouse effects, and maintain or improve soil fertility.

Data availability. Original data are available upon request. Material necessary for this study's findings is presented in the paper.

Author contributions. Kaikuo Wu, Wentao Li, Zhanbo Wei and Zhi Dong conceived and designed the experiments; Yue Meng and Na Lv performed the experiments; Kaikuo Wu analyzed the data; Kaikuo Wu and Lili Zhang wrote the paper and all authors approved submission of the paper. All authors have read and agreed to the published version of the manuscript.

Competing interests. The authors declare that they have no conflict of interest.

Acknowledgements. The authors thank the editors and reviewers for their constructive comments and suggestions.

Financial support. This work was supported by the Strategic Priority Research Program of the Chinese Academy of Sciences (XDA28090200), the National Scientific Foundation Project of China (31971531), the Liaoning "Take the Lead" Project of 2021: Arable Land Productivity Improvement Strategies Development and Promotion (2021JH1/10400039-2), the K.C.Wong Education Foundation (Research and application of environmental-friendly polymer compound fertilizers 2022-5), and the Youth Start-up Fund (2022000008).

Review statement.

References

- Allen, D.E., Kingston, G., Rennenberg, H., Dalal, R.C., and Schmidt, S.:
Effect of nitrogen fertilizer management and waterlogging on nitrous
oxide emission from subtropical sugarcane soils, *Agr. Ecosyst.*
Environ., 136, 209-217, 2010.
- Andren, O., Rajkai, K., and Katterer, T.: Water and temperature dynamics
in a clay soil under winter wheat: influence on straw decomposition
and N immobilization, *Biol. Fert. Soils*, 15, 1-8, 1993.
- Bouman, B.A.M., and Tuong, T.P.: Field water management to save water
and increase its productivity in irrigated lowland rice, *Agr. Water*
Manage., 49, 11-30, 2001.
- Buchen, C., Lewicka-Szczebak, D., Fuß R., Helfrich, M., Flessa, H., and
Well, R.: Fluxes of N₂ and N₂O and contributing processes in
summer after grassland renewal and grassland conversion to maize
cropping on a Plaggic Anthrosol and a Histic Gleysol, *Soil Biol.*
Biochem., 101, 6-19, 2016.
- Carrijo, D.R., Lundy, M.E., and Linquist, B.A.: Rice yields and water use
under alternate wetting and drying irrigation: A meta-analysis, *Field*
Crop. Res., 203, 173-180, 2017.
- Chapuis-Lydie, L., Wrage, N., Metay, A., Chotte, J., and Bernoux, M.:
Soils, a sink for N₂O? A review, *Global Change Biol.*, 13, 1-17,
2007.

- Chen, A., Xie, X., Dorodnikov, M., Wang, W., Ge, T., Shibistova, O., Wei, W., and Guggenberger, G.: Response of paddy soil organic carbon accumulation to changes in long-term yield-driven carbon inputs in subtropical China, *Agr. Ecosyst. Environ.*, 232, 302-311, 2016.
- Chu, G., Chen, T., Wang, Z., Yang, J., and Zhang, J.: Morphological and physiological traits of roots and their relationships with water productivity in water-saving and drought-resistant rice, *Field Crop. Res.*, 162, 108-119, 2014.
- Congreves, K.A., Phan, T., and Farrell, R.E.: A new look at an old concept: using $^{15}\text{N}_2\text{O}$ isotopomers to understand the relationship between soil moisture and N_2O production pathways, *Soil*, 5, 265-274, 2019.
- Fiedler, S.R., Augustin, J., Wrage-Mönnig, N., Jurasinski, G., Gusovius, B., and Glatzel, S.: Potential short-term losses of N_2O and N_2 from high concentrations of biogas digestate in arable soils, *Soil*, 3, 161-176, 2017.
- Huang, W., Wu, J.F., Pan, X.H., Tan, X.M., Zeng, Y.J., Shi, Q.H., Liu, T.J., and Zeng, Y.H.: Effects of long-term straw return on soil organic carbon fractions and enzyme activities in a double-cropped rice paddy in South China, *J. Integr. Agr.*, 20, 236-247, 2021.
- Joergensen, R.G., and Mueller, T.: The fumigation-extraction method to estimate soil microbial biomass: Calibration of the $k(\text{EN})$ value, *Soil*

504 Biol. Biochem., 28(1), 33-37, 1996.
 505 Kritee, K., Nair, D., Zavala-Araiza, D., Proville, J., Rudek, J., Adhya,
 506 T.K., Loecke, T., Esteves, T., Balireddygar, S., Dava, O., Ram, K., S,
 507 R.A., Madasamy, M., Dokka, R.V., Anandaraj, D., Athiyaman, D.,
 508 Reddy, M., Ahuja, R., and Hamburg, S.P.: High nitrous oxide fluxes
 509 from rice indicate the need to manage water for both long- and
 510 short-term climate impacts, P. Natl. Acad. Sci. USA, 115, 9720-9725,
 511 2018.
 512 Ku, H.H., Ryu, J.H., Bae, H.S., Jeong, C., and Lee, S.E.: Modeling a
 513 long-term effect of rice straw incorporation on SOC content and
 514 grain yield in rice field, Arch. Agron. Soil Sci., 65, 1941-1954, 2019.
 515 Lagomarsino, A., Agnelli, A.E., Linqvist, B., Adviento-Borbe, M.A.,
 516 Agnelli, A., Gavina, G., Ravaglia, S., and Ferrara, R.M.: Alternate
 517 wetting and drying of rice reduced CH₄ emissions but triggered N₂O
 518 peaks in a clayey soil of central Italy, Pedosphere, 26, 533-548,
 519 2016.
 520 Lampayan, R.M., Faronilo, J.E., Tuong, T.P., Espiritu, A.J., de Dios, J.L.,
 521 Bayot, R.S., Bueno, C.S., and Hosen, Y.: Effects of seedbed
 522 management and delayed transplanting of rice seedlings on crop
 523 performance, grain yield, and water productivity, Field Crop. Res.,
 524 183, 303-314, 2015.
 525 Li, J.L., Li, Y.e, Wan, Y.F., Wang, B., Waqas, M.A., Cai, W.W., Guo, C.,

526 Zhou, S.H., Su, R.S., Qin, X.B., Gao, Q.Z., and Wilkes, A.:
 527 Combination of modified nitrogen fertilizers and water saving
 528 irrigation can reduce greenhouse gas emissions and increase rice
 529 yield, *Geoderma*, 315, 1-10, 2018a.

530 Li, Z., Li, Z., Muhammad, W., Lin, M.H., Azeem, S., Zhao, H., Lin, S.,
 531 Chen, T., Fang, C.X., Letuma, P., Zhang, Z.X., and Lin, W.X.:
 532 Proteomic analysis of positive influence of alternate wetting and
 533 moderate soil drying on the process of rice grain filling, *Plant*
 534 *Growth Regul.*, 84, 533-548, 2018b.

535 Liang, K., Zhong, X., Huang, N., Lampayan, R.M., Liu, Y., Pan, J., Peng,
 536 B., Hu, X., and Fu, Y.: Nitrogen losses and greenhouse gas
 537 emissions under different N and water management in a subtropical
 538 double-season rice cropping system, *Sci. Total Environ.*, 609, 46-57,
 539 2017.

540 Liao, B., Wu, X., Yu, Y., Luo, S., Hu, R., and Lu, G.: Effects of mild
 541 alternate wetting and drying irrigation and mid-season drainage on
 542 CH₄ and N₂O emissions in rice cultivation, *Sci. Total Environ.*, 698,
 543 134212, 2020.

544 Liu, J., Jiang, B., Shen, J., Zhu, X., Yi, W., Li, Y., and Wu, J.: Contrasting
 545 effects of straw and straw-derived biochar applications on soil
 546 carbon accumulation and nitrogen use efficiency in double-rice
 547 cropping systems, *Agr. Ecosyst. Environ.*, 311, 107286, 2021.

548 Ma, Q., Wu, Z., Shen, S., Zhou, H., Jiang, C., Xu, Y., Liu, R., and Yu, W.:
 549 Responses of biotic and abiotic effects on conservation and supply
 550 of fertilizer N to inhibitors and glucose inputs, *Soil Biol. Biochem.*,
 551 89, 72-81, 2015.

552 Mathieu, O., Henault, C., Leveque, J., Baujard, E., Milloux, M.J., and
 553 Andreux, F.: Quantifying the contribution of nitrification and
 554 denitrification to the nitrous oxide flux using ^{15}N tracers, *Environ.*
 555 *Pollut.*, 144, 933-940, 2006.

556 Naser, H.M., Nagata, O., Tamura, S., and Hatano, R.: Methane emissions
 557 from five paddy fields with different amounts of rice straw
 558 application in central Hokkaido, Japan, *Soil Sci. Plant Nutr.*, 53,
 559 95-101, 2007.

560 Pérez, T., Trumbore, S.E., Tyler, S.C., Davidson, E.A., Keller, M., and de
 561 Camargo, P.B.: Isotopic variability of N_2O emissions from tropical
 562 forest soils, *Global Biogeochem. Cy.*, 14, 525-535, 2000.

563 Said-Pullicino, D., Cucu, M.A., Sodano, M., Birk, J.J., Glaser, B., and
 564 Celi, L.: Nitrogen immobilization in paddy soils as affected by redox
 565 conditions and rice straw incorporation, *Geoderma*, 228, 44-53,
 566 2014.

567 Sun, L., Ma, Y., Li, B., Xiao, C., Fan, L., and Xiong, Z.: Nitrogen
 568 fertilizer in combination with an ameliorant mitigated yield-scaled
 569 greenhouse gas emissions from a coastal saline rice field in

570 southeastern China, *Environ. Sci. Pollut. Res. Int.*, 25, 15896-15908,
571 2018.

572 Tan, X., Shao, D., and Gu, W.: Effects of temperature and soil moisture
573 on gross nitrification and denitrification rates of a Chinese lowland
574 paddy field soil, *Paddy Water Environ.*, 16, 687-698, 2018.

575 Tang, D., and Cheng, Z.: From Basic Research to Molecular Breeding -
576 Chinese Scientists Play A Central Role in Boosting World Rice
577 Production, *Genom. Proteom. Bioinf.*, 16, 389-392, 2018.

578 van Groenigen, J.W., Huygens, D., Boeckx, P., Kuyper, T.W., Lubbers,
579 I.M., Rütting, T., and Groffman, P.M.: The soil N cycle: new insights
580 and key challenges, *Soil*, 1, 235-256, 2015.

581 Verhoeven, E., Decock, C., Barthel, M., Bertora, C., Sacco, D., Romani,
582 M., Sleutel, S., and Six, J.: Nitrification and coupled
583 nitrification-denitrification at shallow depths are responsible for
584 early season N₂O emissions under alternate wetting and drying
585 management in an Italian rice paddy system, *Soil Biol. Biochem.*,
586 120, 58-69, 2018.

587 Vitale, L., Polimeno, F., Ottaiano, L., Maglione, G., Tedeschi, A., Mori,
588 M., De Marco, A., Di Tommasi, P., and Magliulo, V.: Fertilizer type
589 influences tomato yield and soil N₂O emissions, *Plant Soil Environ.*,
590 63, 105-110, 2017.

591 Wang, B., Shen, X., Chen, S., Bai, Y., Yang, G., Zhu, J., Shu, J., and Xue,

Z.: Distribution characteristics, resource utilization and popularizing demonstration of crop straw in southwest China: A comprehensive evaluation, *Ecol. Indic.*, 93, 998-1004, 2018.

Wang, J., Zhao, Y., Zhang, J., Zhao, W., Müller, C., and Cai, Z.: Nitrification is the key process determining N use efficiency in paddy soils, *J. Plant Nutr. Soil Sc.*, 180, 648-658, 2017a.

Wang, J.Y., Jia, J.X., Xiong, Z.Q., Khalil, M.A.K., and Xing, G.X.: Water regime–nitrogen fertilizer–straw incorporation interaction: Field study on nitrous oxide emissions from a rice agroecosystem in Nanjing, China, *Agr. Ecosyst. Environ.*, 141, 437-446, 2011.

Wang, L., Sheng, R., Yang, H., Wang, Q., Zhang, W., Hou, H., Wu, J., and Wei, W.: Stimulatory effect of exogenous nitrate on soil denitrifiers and denitrifying activities in submerged paddy soil, *Geoderma*, 286, 64-72, 2017b.

Wu, K., Zhang, Z., Feng, L., Bai, W., Feng, C., Song, Y., Gong, P., Meng, Y., and Zhang, L.: Effects of corn stalks and urea on N₂O production from corn field soil, *Agronomy-Basel*, 11, 2009, 2021.

Wu, K.K., Gong, P., Zhang, L.L., Wu, Z.J., Xie, X.S., Yang, H.Z., Li, W.T., Song, Y.C., and Li, D.P.: Yield-scaled N₂O and CH₄ emissions as affected by combined application of stabilized nitrogen fertilizer and pig manure in rice fields, *Plant Soil Environ.*, 65, 497-502, 2019.

614 Yano, M., Toyoda S., Tokida T., Hayashi K., Hasegawa T., Makabe A.,
615 Koba K., and Yoshida N.: Isotopomer analysis of production,
616 consumption and soil-to-atmosphere emission processes of N₂O at
617 the beginning of paddy field irrigation, *Soil Biol. Biochem.*, 70,
618 66-78, 2014.

619 Ye, R., and Horwath, W.R.: Influence of rice straw on priming of soil C
620 for dissolved organic C and CH₄ production, *Plant Soil*, 417,
621 231-241, 2017.

622 Yu, C., Xie, X., Yang, H., Yang, L., Li, W., Wu, K., Zhang, W., Feng, C.,
623 Li, D., Wu, Z., and Zhang, L.: Effect of straw and inhibitors on the
624 fate of nitrogen applied to paddy soil, *Sci. Rep-UK*, 10, 21582,
625 2020.

626 Yu, C., Zhang, L., Yang, L., Bai, W., Feng, C., Li, W., Wu, K., Li, D., and
627 Wu, Z.: Effect of a urea and urease/nitrification inhibitor
628 combination on rice straw hydrolysis and nutrient turnover on rice
629 growth, *Bioresources*, 16, 3059-3074, 2021.

630 Zhang, H., Xue, Y.G., Wang, Z.Q., Yang, J.C., and Zhang, J.H.: An
631 Alternate Wetting and Moderate Soil Drying Regime Improves Root
632 and Shoot Growth in Rice, *Crop Sci.*, 49, 2246-2260, 2009.

633 Zhou, Q., Ju, C.X., Wang, Z.Q., Zhang, H., Liu, L.J., Yang, J.C., and
634 Zhang, J.H.: Grain yield and water use efficiency of super rice under
635 soil water deficit and alternate wetting and drying irrigation, *J. Integr.*

636 Agr., 16, 1028-1043, 2017.

637 Zhou, S., Sun, H., Bi, J., Zhang, J., Riya, S., and Hosomi, M.: Effect of
638 water-saving irrigation on the N₂O dynamics and the contribution of
639 exogenous and endogenous nitrogen to N₂O production in paddy soil
640 using ¹⁵N tracing, Soil Till. Res., 200, 104610, 2020.

641 Zhou, Y., Li, X., and Liu, Y.: Cultivated land protection and rational use
642 in China, Land Use Policy, 106, 105454, 2021.

643

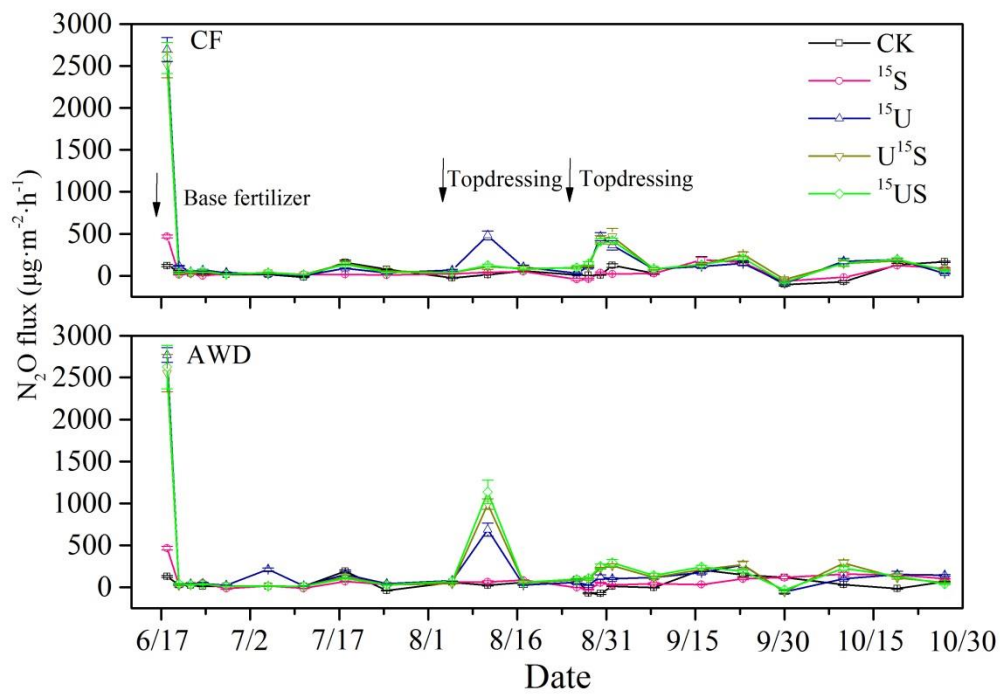
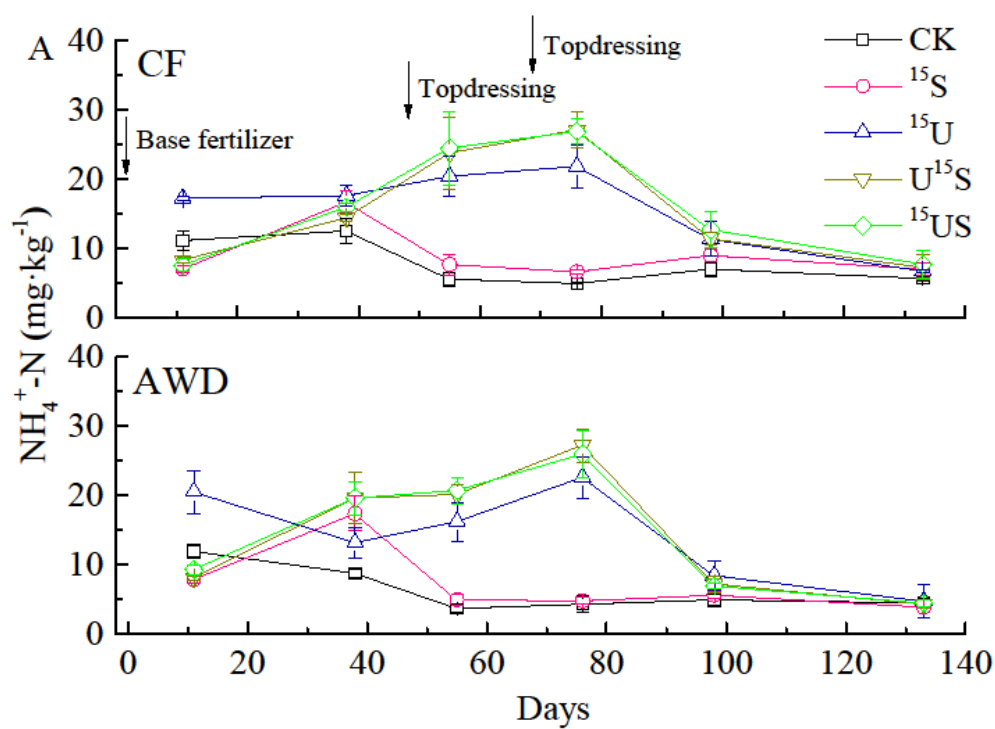
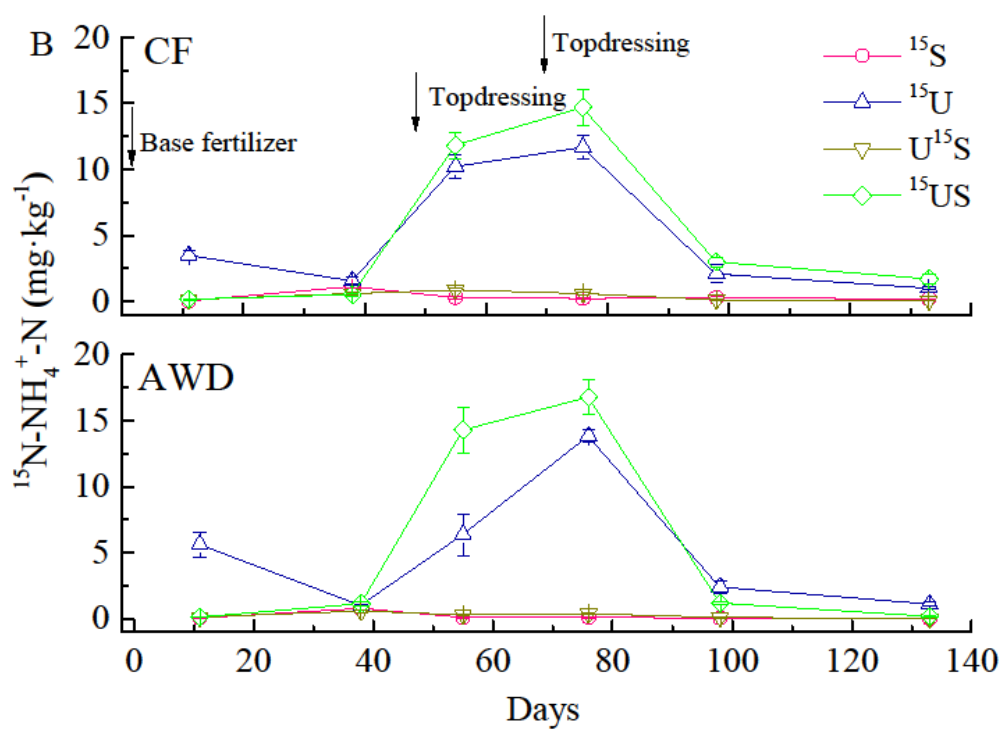


Fig. 1 Effects of different treatments on nitrous oxide (N_2O) flux. Bars represent standard errors (n=3), the same below.



657



658

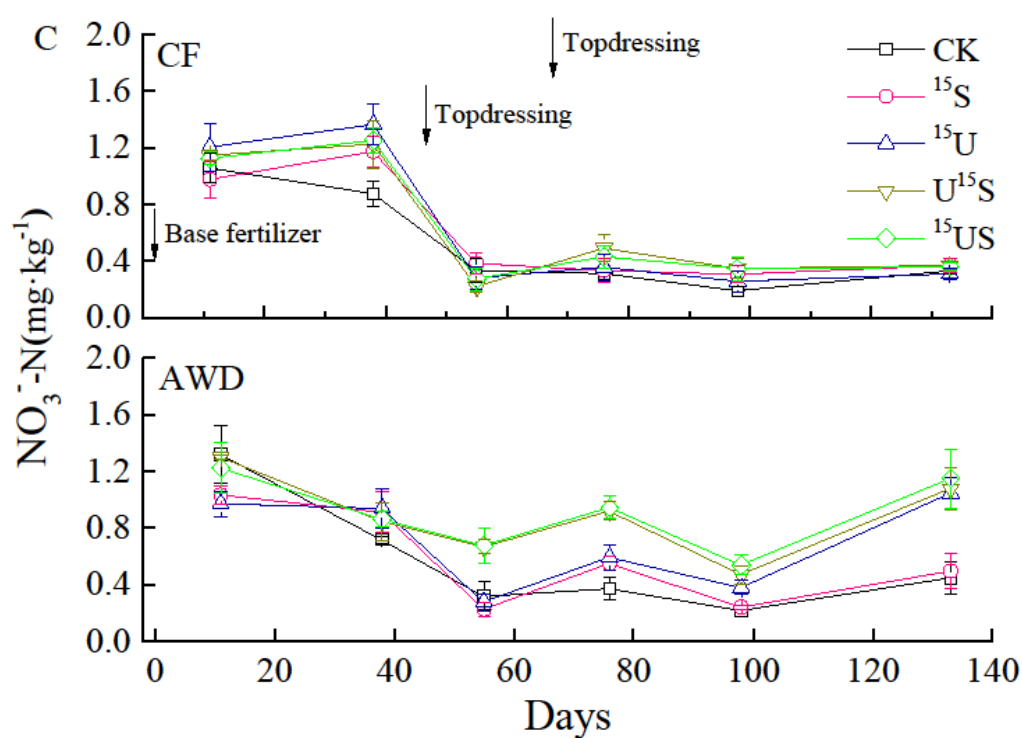


Fig. 2 Changes in soil ammonium nitrogen ($\text{NH}_4^+ \text{-N}$) concentration (A), the contribution of ^{15}N markers to $\text{NH}_4^+ \text{-N}$ (B) and changes in soil nitrate nitrogen ($\text{NO}_3^- \text{-N}$) concentration (C) during the growth period of rice (n=3).

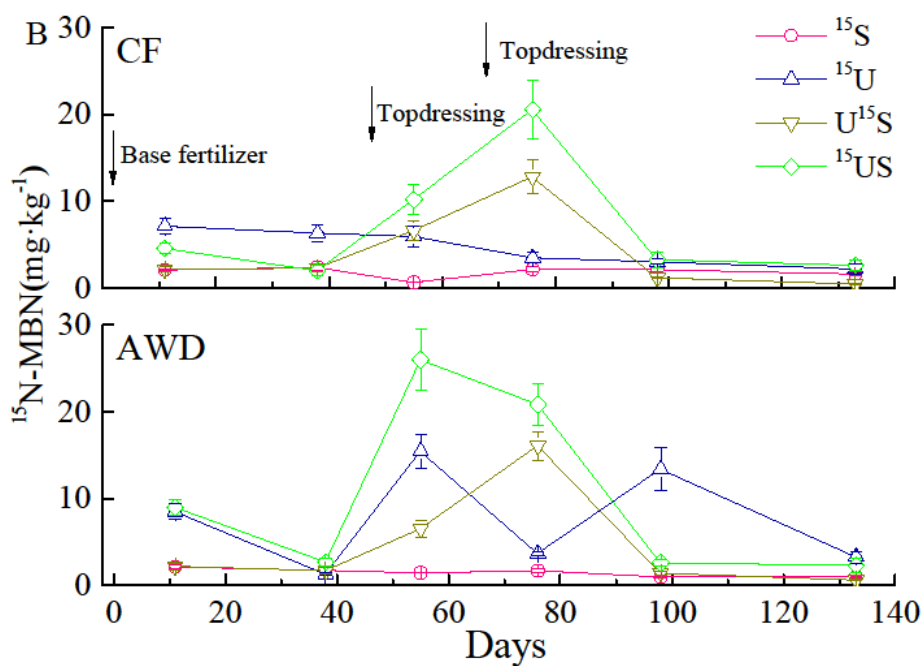
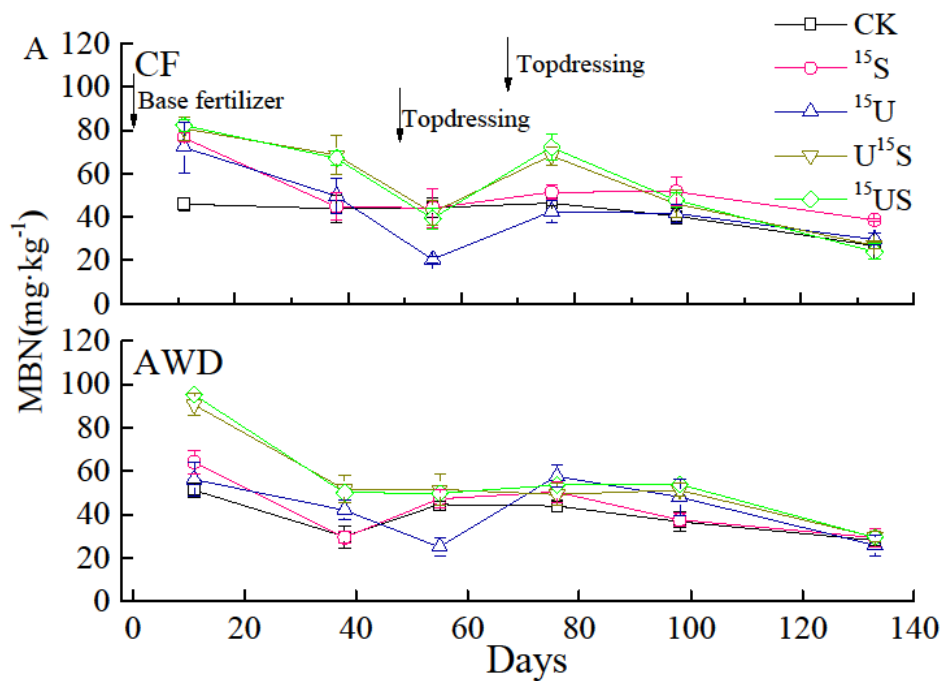


Fig. 3 Changes in the concentration of microbial biomass N (MBN) (A) in the soil during the rice growth period and the contribution of ^{15}N markers to MBN (B) (n=3).

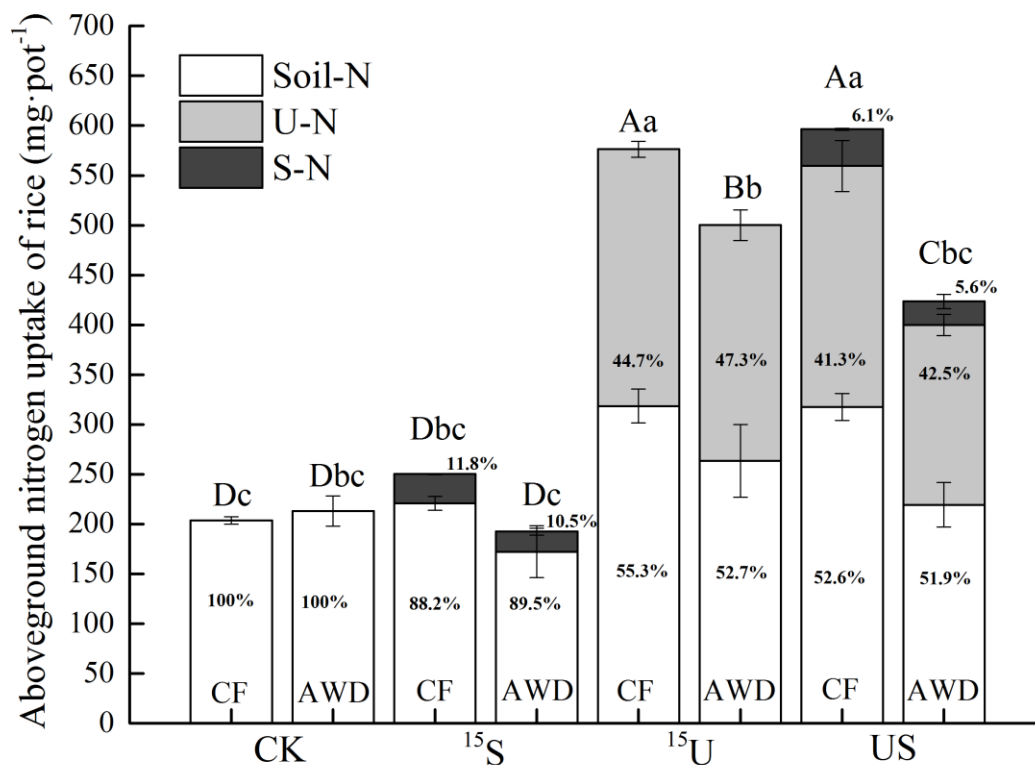


Fig. 4 The source of nitrogen in the aboveground biomass of rice at the maturity stage (n=3). CF: continuous flooding irrigation, AWD: mild alternate wetting and drying irrigation, Soil-N: Soil derived nitrogen, U-N: Urea derived nitrogen, S-N: Rice straw derived nitrogen. Different capital letters indicate significant differences in total nitrogen uptake of rice above ground ($P < 0.05$), and different lower case letters indicate significant differences in soil nitrogen supply ($P < 0.05$).

Table 1 Effects of different treatments on cumulative nitrous oxide (N₂O) emissions, rice aboveground biomass, rice yield and yield-scaled N₂O emission. The values denote means $\pm$ standard errors (n=3). Different lowercase letters indicate significant differences ($P < 0.05$).

Treatments		Cumulative N ₂ O emissions kg ha ⁻¹	Rice aboveground biomass g pot ⁻¹	Rice yield g pot ⁻¹	Yield-scaled N ₂ O emission g kg ⁻¹
CF	CK	1.48 $\pm$ 0.06 ef	37.11 $\pm$ 2.53 f	21.63 $\pm$ 1.81 b	0.24 $\pm$ 0.01 d
	¹⁵ S	1.24 $\pm$ 0.05 f	37.99 $\pm$ 2.69 f	20.91 $\pm$ 1.63 b	0.21 $\pm$ 0.03 d
	¹⁵ U	4.02 $\pm$ 0.30 c	82.54 $\pm$ 10.39 ab	36.56 $\pm$ 2.75 a	0.39 $\pm$ 0.04 c
	U+ ¹⁵ S	3.89 $\pm$ 0.09 c	87.58 $\pm$ 7.70 a	35.98 $\pm$ 1.72 a	0.38 $\pm$ 0.02 c
	¹⁵ U+S	3.76 $\pm$ 0.02 c	80.99 $\pm$ 19.54 abc	35.85 $\pm$ 2.50 a	0.37 $\pm$ 0.03 c
AWD	CK	1.64 $\pm$ 0.15 e	45.31 $\pm$ 3.07 ef	22.92 $\pm$ 1.07 b	0.25 $\pm$ 0.02 d
	¹⁵ S	2.07 $\pm$ 0.17 d	34.78 $\pm$ 4.86 f	20.42 $\pm$ 0.46 b	0.36 $\pm$ 0.02 c
	¹⁵ U	4.48 $\pm$ 0.12 b	64.44 $\pm$ 7.33 bcd	34.81 $\pm$ 1.64 a	0.46 $\pm$ 0.03 b
	U+ ¹⁵ S	5.05 $\pm$ 0.28 a	62.92 $\pm$ 2.49 cde	34.50 $\pm$ 1.76 a	0.53 $\pm$ 0.01 a
	¹⁵ U+S	5.29 $\pm$ 0.25 a	60.44 $\pm$ 6.74 de	34.85 $\pm$ 1.18 a	0.54 $\pm$ 0.03 a

Table 2 Cumulative nitrous oxide (N₂O) emissions in response to irrigation method, nitrogen level, and straw returning. * indicated significant treatment effects within a main category ($P < 0.05$), *** indicated significant treatment effects within a main category ($P < 0.001$).

Factors	Cumulative N ₂ O emissions
Irrigation method (I)	88.576***
Nitrogen level (N)	1525***
Straw (S)	6.393*
I×N	6.275*
I×S	26.288***
N×S	1.426
I×N×S	0.178